17

GLAXO GROUP RESEARCH

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Effect of Ethanol on the Stability of Zantac Syrup

INTRODUCTION

Some time ago, an analysis was carried out to investigate the effect of ethanol on the stability of Zantac syrup[1]. Since only short term data were available at the time (6 to 12 months), no definite conclusions could be drawn, although there was some evidence to suggest that the addition of ethanol improved stability. In 1986, a patent was drawn up to cover this enhanced stability and several definitive studies were set up to assess the stabilising effect of ethanol in the formulation.

To decide whether it is worthwhile pursuing the original patent claim, data from all relevant studies have been collected together for analysis. The data available are summarised below :

1. UK Ingredients

Stability programmes with ethanol :

PR2828 (300ml) - at IN, 4°, 20°, 30°, 37°, 45° C
PR2829 (300ml) - at IN, 4°, 20°, 30°, 37°, 45° C
SP88/078 (300ml) - at IN, 4°, 25°, 30°, 37°, 45° C
SP88/101 (300ml) - at 4°, 25°, 30°, 37°, 45° C
SP87/132 (150ml) - at IN, 4°, 20°, 25°, 30°, 37°, 45° C
SP88/026 (150ml) - at IN, 4°, 25°, 30°, 37°, 45° C

Stability programmes without ethanol :

PR2713 (300ml) - at IN, 4°, 20°, 30°, 37°, 45° C
PR2714 (300ml) - at IN, 4°, 20°, 30°, 37°, 45° C

1 Ref : WDR/051/D6 - analysed by David Robinson (11th Sep. 86)

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34

DEFENDANT'S EXHIBIT

GLAXO WELCOME INC. AND GLAXO GROUP LIMITED v. PHARMADYNE CORPORATION
CIVIL ACTION NO. AND 96-455 HIGHLY CONFIDENTIAL UNDER PROTECTIVE ORDER

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2. US Ingredients
(all at IN, 4°, 20°, 30°, 37°, 45° C)

Stability programmes with ethanol :

PR2834
PR2836
PR2840

Stability programmes without ethanol :

PR2738
PR2739

3. Definitive experiment - Zantac syrup
(all at IN, 4°, 37°, 45° C)

Prog.	ethanol
SP86/121	0%
SP86/122	2.5%
SP86/123	5.0%
SP86/124	7.5%
SP86/125	10.0%

4. Definitive experiment - Zantac solution
(all at IN, 4°, 45°, 65° C)

Prog.	ethanol
SP86/126	0%
SP86/127	7.5%

METHODS

Those studies previously analysed showed that a first order linear degradation model relative to the 4° C data provided a better fit than a model relative to the initial data. In order to use a 4° C model, there must be no degradation at that temperature. This was checked by assessing the change in ranitidine content at 4° C over time and by comparing the average content with that obtained initially.

For the first two groups (ie. UK & US ingredients), an analysis of variance was performed to assess deviations from an Arrhenius model. The data from studies in groups 3 & 4 (ie. definitive experiments) were only recorded at two temperatures (in addition to initial & 4° C) and hence it was not possible to assess deviations from the Arrhenius model. In this situation, the Arrhenius fit is similar to fitting a linear regression between two points. The fitted values from the Arrhenius model (for groups 3 & 4) will therefore be similar to those obtained from separate analyses at each of the two temperatures.

Comparisons between the ethanol and non-ethanol programmes were performed separately on each of the four groups given above.

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RESULTS

In general, the fit was better relative to 4° C than relative to the initial data. Hence for this reason (and also to be consistent with previous analyses), the model relative to 4° C was chosen.

Tests were performed on all the stability programmes to ensure there was no degradation at 4° C. The results of this analysis (given in the Appendix) showed no clear evidence of degradation, allowing the remaining analysis to be carried out relative to 4° C data.

For the first two groups the Arrhenius model provided a reasonable fit to the data from the ethanol programmes, but there were significant deviations from the fit for the programmes without ethanol ($p < 0.05$). Since the Arrhenius model did not fit all the data well and could not be validated for groups 3 & 4, the comparisons between the ethanol and non-ethanol studies were carried out without making the Arrhenius assumption.

A first order linear degradation model was fitted at each of the temperatures required for analysis.

UK Ingredients

The ethanol studies were assessed separately at each temperature. An attempt was then made to pool the data from the six programmes, at 30° & 45° C. The model parameters were found not to differ between programmes at 30° C ($\chi^2 = 2.25$ with 5 df, $p = 0.81$), but there were significant differences at 45° C ($\chi^2 = 28.02$ with 5 df, $p < 0.001$). However, it was possible to pool the ethanol studies at 45° C, by excluding SP88/026 ($\chi^2 = 0.63$ with 4 df, $p = 0.96$).

Studies PR2713 & PR2714 (without ethanol) were not significantly different at both 30° and 45° C ($\chi^2 = 0.69$ & 0.03 with 1 df, $p = 0.41$ & 0.87 respectively) and could therefore, be pooled.

As a result of these findings, the following comparisons were made between the rate constants of the ethanol and non-ethanol studies :

a) PR2828, PR2829, SP88/078, SP88/101, SP87/132 & SP88/026 vs. PR2713 & PR2714 (at 30° C).
The ethanol studies gave a significantly lower rate constant than the non-ethanol studies ($p=0.015$), yielding a longer shelf-life.

b) PR2828, PR2829, SP88/078, SP88/101 & SP87/132 vs. PR2713 & PR2714 (at 45° C).
There was no significant difference between the two groups of studies ($p=0.071$).

c) SP88/026 vs. PR2713 & PR2714 (at 45° C).
SP88/026 with ethanol had a significantly higher rate constant than the non-ethanol studies ($p<0.001$), yielding a shorter shelf-life.

NE

15 May 1990

The results of these analyses are summarised in Table 1.1. Estimates for the rate constant and shelf-life (ie. 95% lower confidence limit on 5% degradation on the initial content) were made for each programme separately and for the combined programmes (Tables 1.2 & 1.3).

Although the Arrhenius model provided a bad fit for the non-ethanol programmes, it was acceptable for the ethanol programmes. Therefore the Arrhenius fit was used to obtain estimates of shelf-life for the ethanol programmes only, at the temperatures of interest (Table 1.4).

US Ingredients

The ethanol studies were assessed separately at each temperature. An attempt was then made to pool the data from the three programmes, at 30° & 45° C. The model parameters were not significantly different between programmes at both 30° & 45° C (χ^2 = 1.91 & 1.61 with 2 df, p = 0.38 & 0.45 respectively), therefore the ethanol studies could be pooled.

Studies PR2738 & PR2739 (without ethanol) were found to be significantly different at both 30° and 45° C (χ^2 = 5.01 & 20.91 with 1 df, p = 0.025 & < 0.001 respectively).

From these results, the following comparisons were made between the rate constants of ethanol and non-ethanol studies :

a) PR2834, PR2836 & PR2840 vs. PR2738 (at 30° C)

b) PR2834, PR2836 & PR2840 vs. PR2739 (at 30° C)

c) PR2834, PR2836 & PR2840 vs. PR2738 (at 45° C)

d) PR2834, PR2836 & PR2840 vs. PR2739 (at 45° C)

The results of these analyses are given in Table 2.1. For all comparisons, the ethanol studies gave a significantly lower rate constant than the non-ethanol studies, yielding a longer shelf-life. Estimates for the rate constant and shelf-life (ie. 95% lower confidence limit on 5% degradation on the initial content) were made for each programme separately and for the combined programmes (Tables 2.2 & 2.3).

Although the Arrhenius model provided a bad fit for the non-ethanol programmes, it was acceptable for the ethanol programmes. Therefore the Arrhenius fit was used to obtain estimates of shelf-life for the ethanol programmes only, at the temperatures of interest (Table 2.3).

Zantac Syrup

The data from these five programmes were examined at 37° & 45° C, to determine the relationship between the rate constant and the concentration of ethanol in the syrup formulation. The results of this test of trend are summarised in Table 3.1. There was a significant decreasing relationship between the rate constant and ethanol concentration at

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45° C, but not at 30° C. Estimates for the shelf-life (ie. 95% lower confidence limit on 5% degradation on the label claim) were made at 37° & 45° C, for each of the five programmes (Table 3.2).

Zantac Solution

A comparison between the two programmes was made at 45° C, to assess the effect of ethanol in solution. The ethanol study (SP86/127) gave a significantly lower rate constant than the non-ethanol study (p=0.001), yielding a longer shelf-life. The results of this test are given in Table 4.1. Estimates for the shelf-life (ie. 95% lower confidence limit on 5% degradation on the label claim) were made for the two programmes at 45° C (Table 4.2).

Graphs of ranitidine content relative to 4° C data are given for each stability programme. The fitted lines are obtained from the first order linear degradation model at each temperature. The points are the observed content expressed as a percentage of the fitted content at 4° C. Also given are the graphs obtained from the Arrhenius fit for the UK & US ethanol programmes.

CONCLUSION

In order to be consistent with previous analyses, the first order linear degradation model relative to the 4° C data was fitted. This was possible as there was no real evidence of degradation at 4° C. The Arrhenius model provided a bad fit to the data from non-ethanol programmes and consequently, was not used in the ethanol vs. non-ethanol comparisons.

There were significant differences in rate constant between the ethanol and non-ethanol groups at 30° C, giving longer shelf-lives for both UK and US ingredients. At 45° C, the shelf-lives were longer for US ethanol programmes. There was no significant difference in rate constant between the ethanol and non-ethanol groups (excluding SP88/026) at 45° C, for UK ingrdients.

The shelf-lives at 45° C increased as the ethanol concentration increased for Zantac syrup, but this was not the case at 37° C. For the Zantac solution programmes, the shelf-life at 45° C was longer for the ethanol than for the non-ethanol group.

N. Elahi.

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Table 1.1 - Analysis of rate constants for UK ingredients

Test	Ethanol[$]	No Ethanol[$]	χ^2_1	p value
a) 30°	26.2	27.6	5.93	0.015*
b) 45°	172.2	166.0	3.27	0.071
c) 45°	227.0	166.0	41.13	< 0.001*

[$] - tabulated values are geometric mean rate constants of each group multiplied by 10^4.
* - significant at the 5% level.

Table 1.2 - Estimates of rate constant (K) obtained from single temperature analysis

UK Ingredients		K x 10^4 30°C	K x 10^4 45°C
Ethanol	PR2828	26.7	176.7
	PR2829	25.3	160.5
	SP88/078	27.0	169.3
	SP88/101	26.7	176.9
	SP87/132	26.4	178.3
	SP88/026	24.9	226.6
	Mean[$]	26.2	172.2[x]
No Ethanol	PR2713	28.1	165.4
	PR2714	27.0	166.6
	Mean[$]	27.6	166.0

[$] - these are geometric mean rate constants of each group multiplied by 10^4.
[x] - this geometric mean was calculated excluding programme SP88/026.

NE

15 May 1990

Table 1.3 - Estimates of shelf-life* in months obtained from single temperature analysis

UK Ingredients		Temperature (° C)			
		20	25	30	45
Ethanol	PR2828	54.0	-	18.4	2.5
	PR2829	54.6	-	19.3	2.4
	SP88/078	-	30.7	16.4	2.7
	SP88/101	-	39.0	17.5	2.8
	SP87/132	40.7	30.5	17.4	2.4
	SP88/026	-	39.7	17.5	2.1
	Combinec	-	-	19.1	2.8x
No Ethanol	PR2713	72.6	-	17.6	3.0
	PR2714	81.5	-	17.7	2.9
	Combined	-	-	17.9	3.0

* - defined as the lower 95% confidence limit on 5% degradation on the initial content.
x - this combined estimate of shelf-life was made excluding programme SP88/026.

Table 1.4 - Estimates of shelf-life* in months obtained from the Arrhenius model

UK Ingredients		Temperature (°C)			
		20	25	30	45
Ethanol	PR2828	65.3	34.3	18.3	2.7
	PR2829	59.9	33.5	18.9	3.1
	SP88/078	56.6	30.7	16.9	3.0
	SP88/101	66.2	34.7	18.5	2.8
	SP87/132	64.3	33.5	17.7	2.6
	SP88/026	95.2	43.2	20.1	2.1

* - defined as the lower 95% confidence limit on 5% degradation on the initial content

NE

15 May 1990

Table 2.1 - Analysis of rate constants for US ingredients

Test	Ethanol[$]	No Ethanol[$]	X_1^2	p value
a) 30°	24.8	39.4	159.20	< 0.001*
b) 30°	24.8	34.3	25.40	< 0.001*
c) 45°	162	269	81.18	< 0.001*
d) 45°	162	200	9.00	0.003*

[$] - tabulated values are geometric mean rate constants of each group multiplied by 10^4.
* - significant at the 5% level.

Table 2.2 - Estimates of rate constant (K) obtained from single temperature analysis

US Ingredients		K x 10^4 30°	K x 10^4 45°
Ethanol	PR2834	24.7	155.0
	PR2836	26.3	160.1
	PR2840	23.6	170.4
	Mean[$]	24.8	162.0
No Ethanol	PR2738	39.4	268.9
	PR2739	34.3	200.3

[$] - these are geometric mean rate constants of each group multiplied by 10^4.

NE

15 May 1990

Table 2.3 - Estimates of shelf-life* in months from single temperature analysis

US Ingredients		Temperature (° C)		
		20	30	45
Ethanol	PR2834	65.7	19.5	2.9
	PR2836	59.9	17.0	2.9
	PR2840	71.1	20.8	2.8
	Combined	-	20.6	3.0
No Ethanol	PR2738	72.2	12.5	1.8
	PR2739	53.8	13.6	2.3

* - defined as the lower 95% confidence limit on 5% degradation on the initial content.

Table 2.4 - Estimates of shelf-life* in months obtained from the Arrhenius model

US Ingredients		Temperature (°C)			
		20	25	30	45
Ethanol	PR2834	66.3	34.9	18.7	3.0
	PR2836	61.2	32.8	17.9	3.0
	PR2840	78.2	39.3	20.1	2.9

* - defined as the lower 95% confidence limit on 5% degradation on the initial content

NE

15 May 1990

GLAXO WELCOME INC. AND GLAXO GROUP LIMITED v. PHARMADYNE CORPORATION
CIVIL ACTION NO. AND 96-455 HIGHLY CONFIDENTIAL UNDER PROTECTIVE ORDER

Y009651

Table 3.1 - Analysis of rate constants for Zantac syrup

	Concentration of Ethanol[$]						
	0%	2.5%	5%	7.5%	10%	χ^2	p value
37°	72.7	56.8	61.5	59.6	60.5	0.83	0.361
45°	231	206	204	187	173	23.45	< 0.001*

$ - tabulated values are geometric mean rate constants of each group multiplied by 10^4.
* - significant at the 5% level.

Table 3.2 - Estimates of shelf-life# in months from single temperature analysis

Ethanol Concentration	Zantac Syrup	Temperature (° C)	
		37	45
0%	SP86/121	5.9	2.1
2.5%	SP86/122	7.2	2.4
5.0%	SP86/123	7.6	2.4
7.5%	SP86/124	7.7	2.6
10.0%	SP86/125	6.4	2.7

- defined as the lower 95% confidence limit on 5% degradation on the initial content.

NE

15 May 1990

Table 4.1 - Analysis of rate constants for Zantac solution

	Ethanol[a]	No Ethanol[a]	X_1^2	p value
45°	217	262	10.48	0.001*

[a] - tabulated values are geometric rate constants of each group multiplied by 10^4.
* - significant at the 5% level.

Table 4.2 - Estimates of shelf-life# in months from single temperature analysis

Ethanol Concentration	Zantac Solution	Temperature (° C)
		45
0%	SP86/126	1.8
7.5%	SP86/127	2.2

- defined as the lower 95% confidence limit on 5% degradation on the initial content.

NE

15 May 1990

APPENDIX

In order to check that there was no degradation at 4° C, the 4° data were compared with the initial ranitidine content using an analysis of variance.

UK Ingredients

Table A1 gives the geometric mean content initially and for all storage times at 4° C. None of the UK programmes gave significant decrease over time ($p>0.05$). Only studies PR2713 & PR2714 showed evidence of a significant difference between the initial content and the average at 4° C ($p<0.001$). These significant results were due to the low assay readings at 36 months and the appreciable drop in ranitidine content from the initial to the 28 day assay.

US Ingredients

Table A2 gives the geometric mean content initially and for all storage times at 4° C. Programmes PR2834 & PR2836 gave no significant decrease over time ($p>0.05$) and a significant difference between the initial content and the average at 4° C ($p<0.01$). For PR2834, this was mainly due to the low 28 day assay value. Programme PR2840 gave a significant decrease over time ($p<0.01$), but no significant difference between the initial content and the average at 4° C ($p>0.05$). This significant result was due to the extremely high content observed at day 28. Programme PR2738 gave no significant decrease over time ($p>0.05$) and a significant difference between the initial content and the average at 4° C ($p<0.001$). Programme PR2739 gave a significant decrease over time ($p<0.001$), but there was no significant difference between the initial content and the average at 4° C ($p>0.05$). Most of the 4° C assay values for PR2739 were greater than the initial content.

Zantac Syrup

Table A3 gives the geometric mean content initially and for all storage times at 4° C. Only study SP86/122 showed evidence of a significant decrease over time ($p<0.05$), but in this case, most of the 4° C assay values were greater than the initial content. None of the studies gave a significant difference between the initial content and the average at 4° C ($p>0.05$).

Zantac Solution

Table A4 gives the geometric mean content initially and for all storage times at 4° C. Only programme SP86/126 showed evidence of a significant decrease over time ($p<0.05$). This was due to the high assay value obtained at 21 days, which was appreciably greater than the initial value. Neither programme gave a significant difference between the initial content and the average content at 4° C ($p>0.05$).

SUMMARY

The use of 4° C data seemed well justified for programmes containing ethanol. The use is equivocal for non-ethanol, particularly because of the low values observed at 36 months for both UK and US ingredients. However, in our judgement, the increase in precision attained by taking account of the 4° C data, outweighs any possible bias introduced.

NE

15 May 1990

Table A1 - Geometric mean ranitidine content of UK ingredients at 4° C

UK Ingredients		Storage Time (months)										
		IN	0.9	3	6	9	12	18	24	29	36	39
Ethanol	PR2828	149.4	149.0	147.2	148.3	-	149.0	147.7	145.9	149.7	149.9	149.9
	PR2829	148.4	149.7	146.6	149.0	-	148.6	147.4	148.2	149.5	149.5	148.5
	SP88/078	152.6	151.6	153.3	151.9	151.9	152.3	151.6	-	-	-	-
	SP88/101	-	151.1	151.5	151.5	151.2	151.4	-	-	-	-	-
	SP87/132	150.7	148.9	150.7	151.5	-	151.7	151.9	150.4	-	-	-
	SP88/026	150.5	151.0	150.2	150.9	-	151.1	150.0	-	-	-	-
No Ethanol	PR2713	149.4	146.7	147.9	147.7	-	148.1	-	147.6	-	145.4	-
	PR2714	148.8	147.5	147.8	146.9	-	148.4	-	148.1	-	145.4	-

Table A2 - Geometric mean ranitidine content of US ingredients at 4° C

US Ingredients		Storage Time (months)										
		IN	0.9	3	6	7	10	12	18	24	36	38
Ethanol	PR2834	150.6	148.0	150.8	-	150.0	150.0	149.5	150.0	149.5	-	-
	PR2836	151.0	150.5	150.0	-	151.0	150.0	151.0	151.0	149.0	-	150.0
	PR2840	150.7	153.0	150.0	-	150.0	150.0	150.5	149.0	148.5	-	149.3
No Ethanol	PR2738	146.3	145.2	146.5	146.2	-	-	144.0	-	144.5	145.0	-
	PR2739	146.9	148.4	147.8	148.1	-	-	147.5	-	147.5	144.0	-

NE

15 May 1990

Table A3 - Geometric mean ranitidine content of Zantac syrup at 4° C

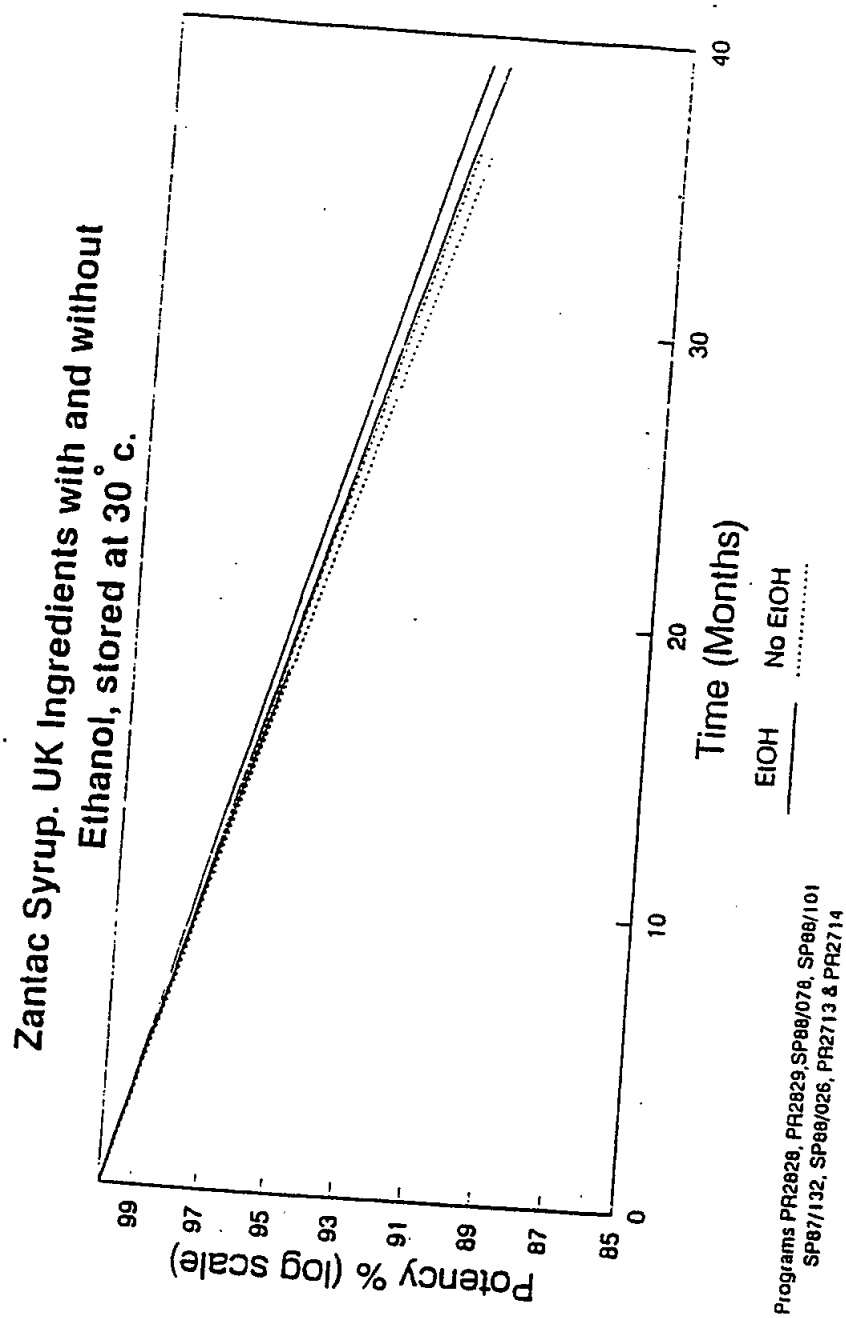
Ethanol Concentration	Zantac Syrup	Storage Time (months)					
		IN	0.7	2	3	6	9
0%	SP86/121	150.1	149.0	150.8	149.5	150.2	149.0
2.5%	SP86/122	149.8	150.2	151.8	149.8	150.0	149.0
5.0%	SP86/123	149.4	148.9	151.1	149.4	150.2	148.9
7.5%	SP86/124	150.4	148.6	150.9	149.4	149.2	149.1
10.0%	SP86/125	150.3	149.9	148.1	149.2	150.1	150.4

Table A4 - Geometric mean ranitidine content of Zantac solution at 4° C

Ethanol Concentration	Zantac Solution	Storage Time (months)					
		IN	0.7	2	3	6	9
0%	SP86/126	149.7	151.7	149.6	150.7	149.7	148.4
7.5%	SP86/127	150.0	150.0	147.6	149.6	149.8	149.5

NE

15 May 1990



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CIVIL ACTION NO. AND 96-455 HIGHLY CONFIDENTIAL UNDER PROTECTIVE ORDER

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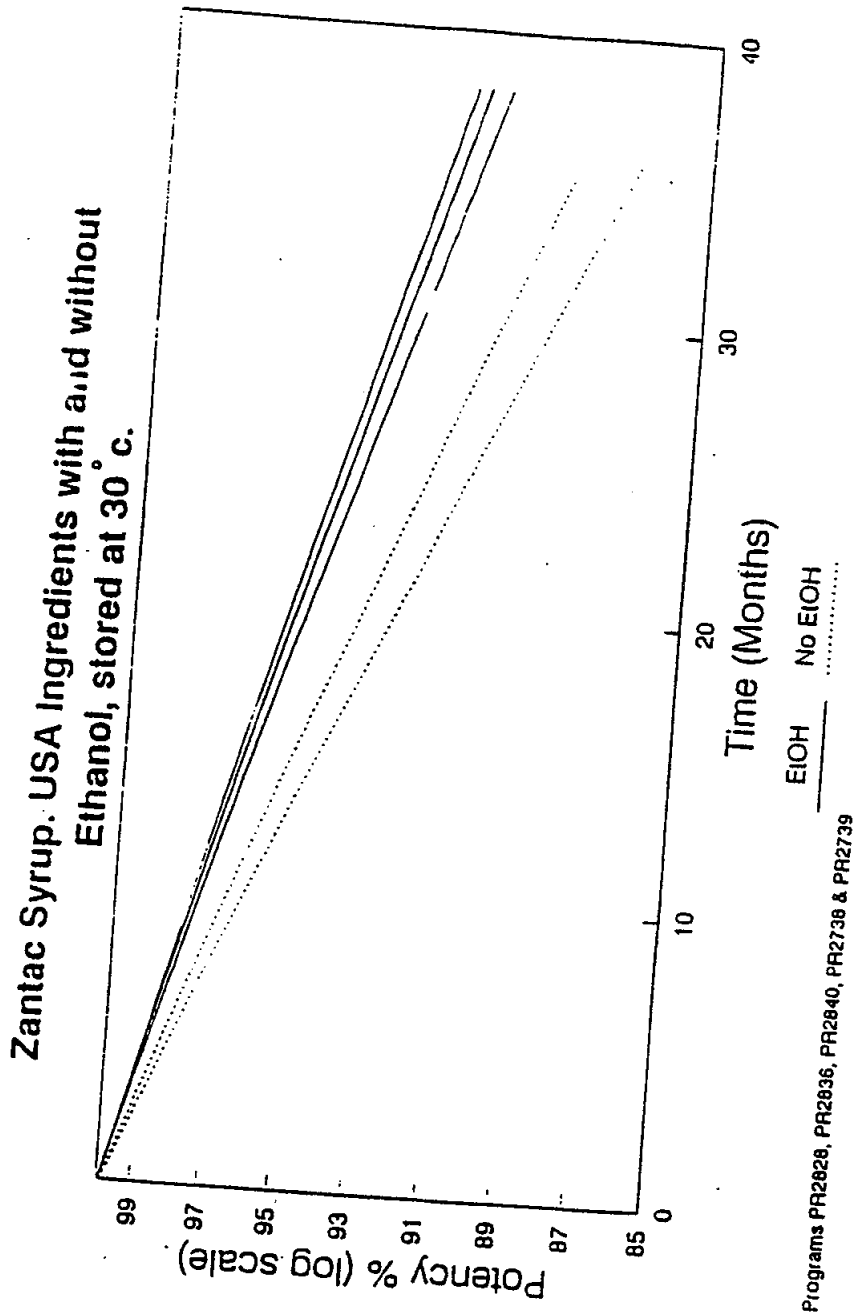

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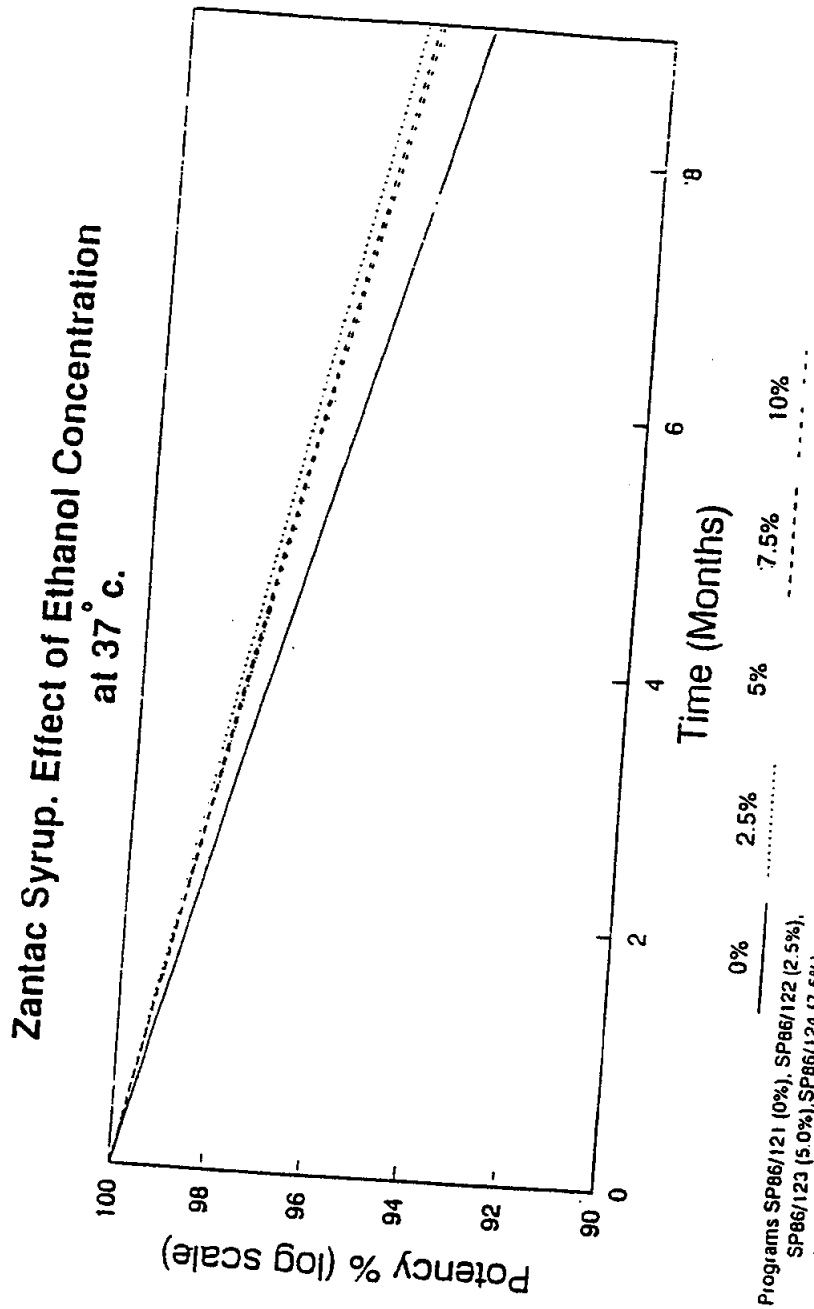

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CIVIL ACTION NO. AND 96-455 HIGHLY CONFIDENTIAL UNDER PROTECTIVE ORDER

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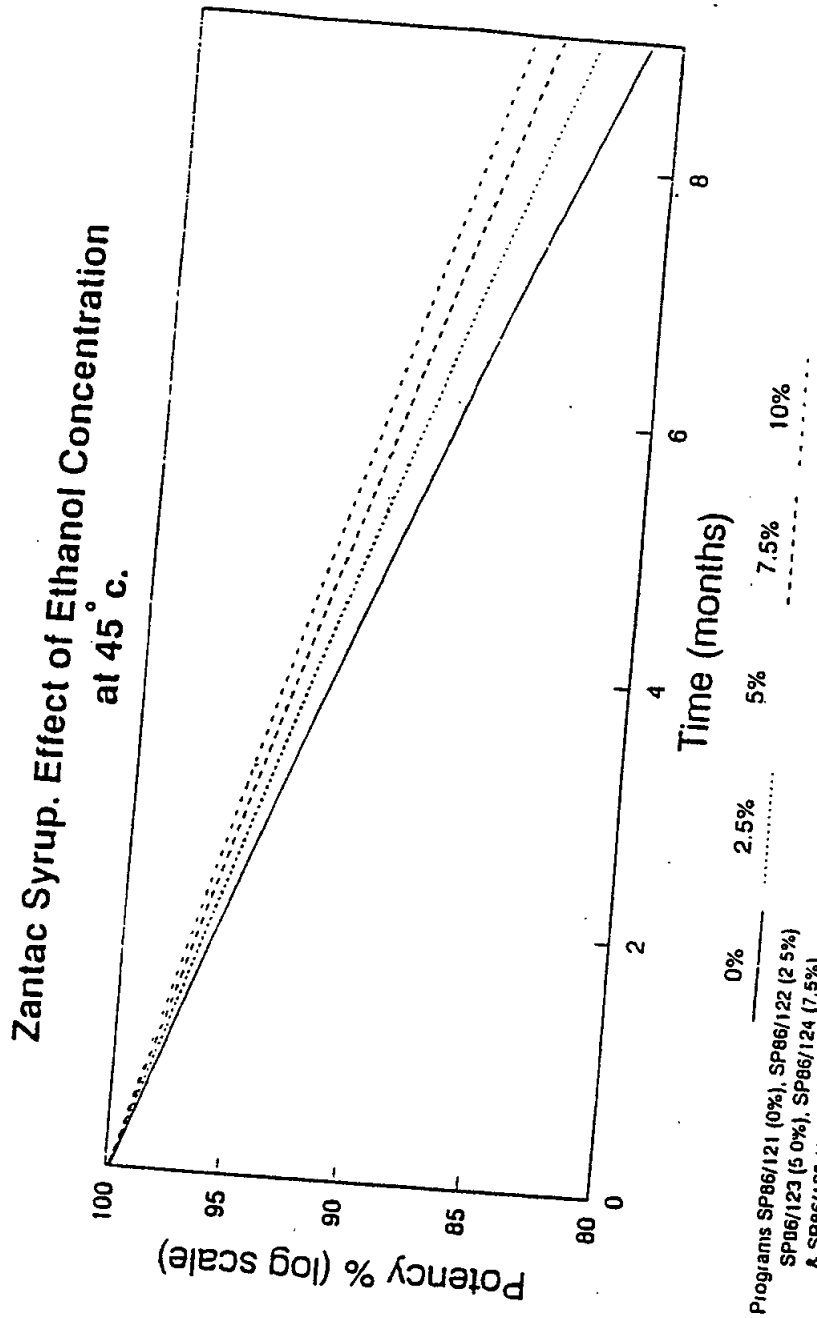

GLAXO WELCOME INC. AND GLAXO GROUP LIMITED v. PHARMADYNE CORPORATION
CIVIL ACTION NO. AMD 96-455 HIGHLY CONFIDENTIAL UNDER PROTECTIVE ORDER

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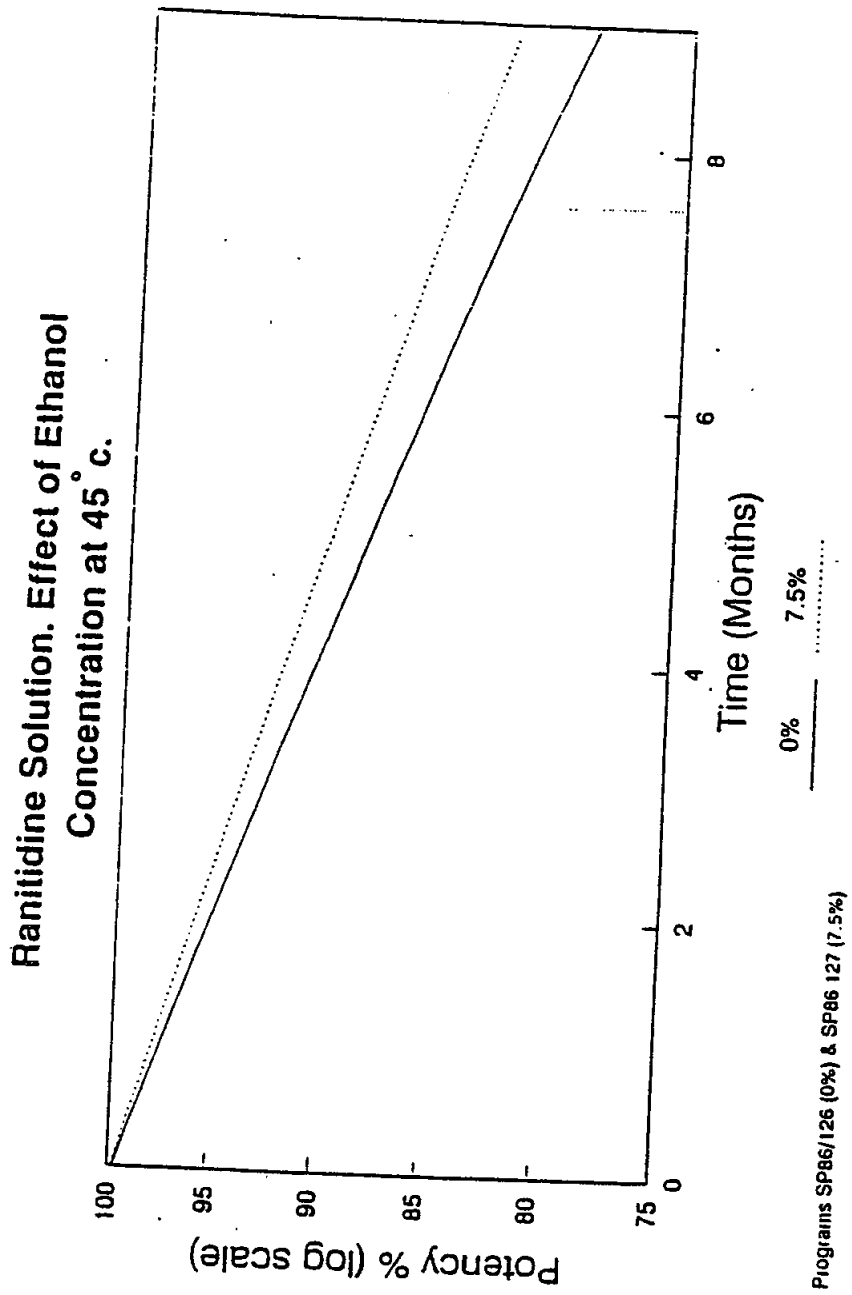
Ranitidine Solution. Effect of Ethanol Concentration at 45°c.
Potency % (log scale)
100
95
90
85
80
75
0
2
4
6
8
Time (Months)
0%
7.5%
Programs SP86/126 (0%) & SP86 127 (7.5%)